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Genetic variations in the IDUA gene in Tunisian MPS I families: Identification of a novel microdeletion disrupting substrate binding and structural insights

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ABSTRACT

Background: Mucopolysaccharidosis type I (MPS I) is a lysosomal storage disorder caused by a deficiency in alpha-L-iduronidase (IDUA), leading to the accumulation of glycosaminoglycans. MPS I presents with a broad spectrum of clinical phenotypes, ranging from severe to mild. This study aimed to identify genetic mutations in the IDUA gene among Tunisian families and assess their structural and functional implications.

Patients and methods: Genomic DNA was extracted from blood samples of four patients including two siblings from three Tunisian families. Polymerase chain reaction (PCR) followed by Sanger sequencing was performed to identify mutations in the IDUA gene. Bioinformatics tools, including the SWISS-MODEL server and DynaMut, were used for structural modeling and to predict the impact of the mutations on protein stability and flexibility.

Results: Two mutations in the IDUA gene were identified. A novel deletion mutation p.His356_Gln362del was discovered in two patients with severe MPS I phenotypes, while a previously reported missense mutation p.Pro533Arg was found in two patients with intermediate and mild phenotypes. Structural analysis revealed that the novel deletion disrupts the protein's substrate-binding site. This deletion causes structural deformation and leads to the elimination of the substrate binding site, resulting in a complete loss of enzymatic activity.

The missense mutation p.Pro533Arg affects the stability and flexibility of the protein, likely reducing substrate affinity. This substitution results in the introduction of a bulkier amino acid, requiring more space in the contact region between the β-sheet structure and the substrate-bound helix.

Conclusion: This study reports a novel deletion mutation in the IDUA gene in Tunisian MPS I patients, alongside a previously described mutation. The findings enhance understanding of the molecular basis of MPS I and provide insights into the structural effects of these mutations, which could aid in future diagnosis and therapeutic strategies. Future studies should explore the prevalence of the reported mutations in larger cohorts and investigate targeted therapies, such as pharmacological chaperones, to rescue enzymatic activity in patients carrying such mutations.

1. Introduction

Mucopolysaccharidosis type I (MPS I) is a rare but severe autosomal recessive lysosomal storage disorder caused by a deficiency in the enzyme alpha-L-iduronidase (IDUA), which plays a crucial role in the degradation of glycosaminoglycans (GAGs), specifically dermatan sulfate and heparan sulfate. The accumulation of these GAGs in various tissues and organs leads to a progressive, multisystemic disorder

Abbreviations: MPS I, Mucopolysaccharidosis type I; IDUA, alpha-L-iduronidase; DNA, deoxyribonucleic acid; MRI, Magnetic Resonance Imaging; HH, hypothalamic-pituitary.

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characterized by a wide range of clinical manifestations, including skeletal deformities, organomegaly, corneal clouding, and, in severe cases, neurological impairment. The clinical presentation of MPS I is highly heterogeneous, ranging from the severe Hurler syndrome (MPS IH) to the milder Scheie syndrome (MPS IS), with an intermediate form, Hurler-Scheie syndrome (MPS IH/S), manifesting features of both. The severity and progression of the disease are largely determined by the level of residual IDUA enzymatic activity, which is directly influenced by mutations in the IDUA gene [1].

The IDUA gene, located on chromosome 4p16.3, spans 19 kb and comprises 14 exons that encode a 653-amino-acid protein [2,3]. Over 300 mutations in the IDUA gene have been identified to date, including missense, nonsense, splicing, insertions, deletions, and large rearrangements, which contribute to the broad phenotypic spectrum observed in MPS I [4]. However, the mutational landscape of MPS I varies significantly across different populations, highlighting the importance of studying specific regional mutations to improve diagnosis, genetic counseling, and treatment strategies.

In Tunisia, the incidence of MPS I is estimated to be higher than in other parts of the world, likely due to the high rate of consanguinity, which is around 32 % nationally and can exceed 60 % in rural areas. Consanguineous marriages, particularly between first cousins, are a well-known risk factor for the occurrence of autosomal recessive genetic disorders such as MPS I [5]. This elevated prevalence underscores the need for systematic genetic screening and early diagnosis of MPS I in Tunisian patients to provide timely and appropriate therapeutic interventions, including enzyme replacement therapy and hematopoietic stem cell transplantation.

Despite the progress made in identifying MPS I-related mutations in Tunisia, the genetic profile of this disorder remains incompletely characterized. In particular, the identification of novel mutations and their structural and functional consequences is critical for advancing our understanding of the disease and improving patient outcomes. In this context, the aim of the present study is twofold: (1) to identify new and previously described mutations in the IDUA gene among Tunisian MPS I patients, and (2) to assess the structural and functional impact of these mutations on the IDUA protein using bioinformatics tools.

2. Patients and methods

2.1. Patients

The present study included four patients including two siblings (P1, P2, P3, and P4) from three unrelated families (F1, F2, F3) originating from three distinct regions: Mahres and Skhira (both within Sfax governorate), and Kairouan.

The patients were evaluated through a series of assessments, including detailed medical history, physical examination, routine haematological and biochemical tests and measurement of leukocyte IDUA activity. Subsequently, genetic testing was conducted to explore potential pathogenic mutations associated with MPS. Family histories and main clinical data are reported in Table 1.

The Ethics Committee of the Farhat Hached Hospital (Sousse, Tunisia) approved this study and, prior to collecting blood samples, the families supplied informed consent. All procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 and approved by the Ethics Committees of the respective Tunisian hospitals.

2.1.1. Family 1

Patient 1, aged 5, was born from a consanguineous union at the 4th degree (Fig. 1). Since his birth in 2019, this patient has been under medical supervision due to a complex malformation syndrome. Investigations included a karyotype analysis and molecular assessment of exon 11 of the fibroblast-growth factor receptor 3 (FGFR 3) gene, prompted by suspicion of achondroplasia. Throughout 2019, the patient presented multiple clinical concerns, including bilateral corneal opacification, untreated spina bifida, kyphoscoliosis, suspected pancreatitis, umbilical hernia, bronchiolitis, and prolonged febrile episodes.

In February 2021, hospital admission was necessitated by abdominal distention. Given the constellation of symptoms, encompassing notable facial dysmorphia, kyphoscoliosis, recurrent otolaryngology infections, umbilical hernia, and developmental delay, a diagnosis of MPS I with an intermediate phenotype was contemplated.

Table 1
Clinical and phenotypic data on the patients studied.

Families	F1	F2	F3	
Origin	Mahres, Sfax	Skhira, Sfax	Kairouan	
Patients	P1	P2	P3	P4
Age	5 years	5 years	Died at the age of 6	Died at the age of 5
Age at onset of symptoms	3 years	3 years	1 year	1 year
Gender	male	male	female	male
Consanguinity	4th degree	4th degree	4th degree	4th degree
Mental retardation	+	–	++	++
Growth retardation	+	+	++	++
Facial dysmorphia	+	+	++	++
Corneal opacities	+	+	++	++
Skeletal deformity	+	–	++	++
Hepatosplenomegaly	+	+	++	++
Umbilical hernia	+	+		
Phenotype	Intermediate	attenuated	Severe	Severe

(+): present; (−): absent; (++): severe.

2.1.2. Family 2

Patient 2 in the second family, aged 5, was born from a consanguineous marriage at the 4th degree (Fig. 1) and had a sibling who succumbed at 6 months old due to congenital heart disease. Since his birth, Patient 2 has been hospitalized due to a maternal-fetal infection and experienced severe bronchiolitis at 3 months of age. In 2019, the patient presented with abdominal distention and respiratory distress. Abdominal ultrasound revealed hepatosplenomegaly, calcific microstones in the right middle region, and mild bilateral pyelectasis.

Further investigations included cerebral magnetic resonance imaging (MRI) focusing on the hypothalamic-pituitary (HH) axis, revealing abnormalities in the white matter, micro-cavities, and enlarged Virchow-Robin spaces. Additionally, distinctive features such as a flared appearance of the sellaturcica, odontoid hypoplasia, and thickening of the transverse ligament were indicative of mucopolysaccharidosis. Ear, nose, and throat examination unveiled mixed hearing loss in the right ear, while ophthalmological assessment identified epithelial and stromal corneal dystrophy. Other notable clinical features included coarse facial features, hepatosplenomegaly, and an umbilical hernia. Based on this characteristic clinical profile, suspicion arose regarding MPS I with a mild phenotype in Patient 2.

2.1.3. Family 3

In this family, siblings (Patients 3 and 4) born from a consanguineous union at the 4th degree, passed away toward of the end 2018 (Fig. 1). The eldest sibling (Patient 3) succumbed at the age of 6, displaying symptoms suggestive of MPS I. Clinical manifestations emerged at 1 year of age, characterized by facial dysmorphia featuring coarse features, thickened skin, macroglossia, and hepatosplenomegaly. Subsequently, the patient developed gingival hypertrophy, dental spacing, corneal opacities, joint stiffness, and lumbar dorsal kyphosis.

The younger sibling, Patient 4, a male, passed away at the age of 5, one-year junior to his sister. His clinical presentation mirrored that of his elder sister (Patient 3), encompassing similar symptoms and signs.

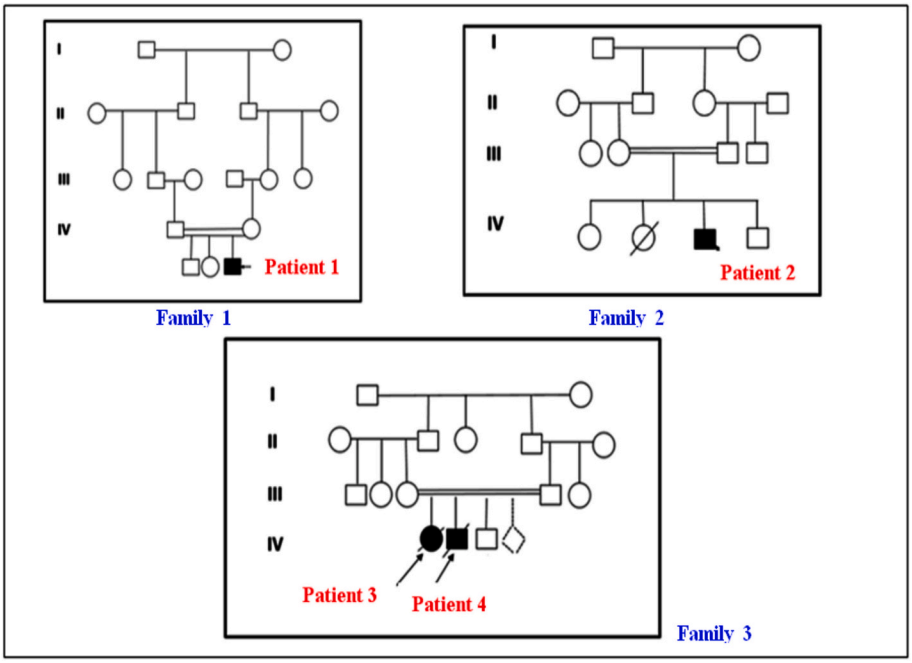

Fig. 1. Pedigrees of the MPS I Tunisian families.
The arrows indicate the analyzed patients in each family. Squares represent males, and circles represent females. Filled symbols denote affected individuals, while open symbols indicate unaffected individuals.

2.2. Laboratory analysis

This biological study was carried out in the biochemistry laboratory at Farhat Hached Hospital, Sousse, Tunisia. The aim of this analysis was to quantitatively measure residual IDUA enzyme activity in patient leukocytes, which serves as a key diagnostic parameter for MPS I. 15 µl of leucocyte suspension was added to 20 µl of substrate "4 methyl-umbelliferyl- α-L-iduronide", 20 µl of 0.2 M formate buffer and 5 µl of distilled water. The reaction mixture and a blank tube were incubated at 37 °C for 2 h. Finally, the reaction was stopped by adding 1.5 ml of 0.5 M bicarbonate-carbonate buffer at pH 10.6. An immediate reading was taken at a wavelength of 660 nm by comparison with a crystallized phenol standard.

2.3. Mutational analysis

Genomic DNA was extracted from peripheral blood samples using the standard salting-out method. For comprehensive mutation screening, we sequenced all 14 coding exons and flanking splice sites of the IDUA gene (NM_000203.4). as described previously [6]. PCR products were then purified and subjected to Sanger sequencing. Sequencing was performed on an Applied Biosystems SeqStudio Genetic Analyzer, following the manufacturer's protocol and as described previously [7]. The resulting sequences were analyzed using sequence alignment software to compare the patients' DNA with the reference sequence.

2.4. In silico analysis

The 3D structure of the Alpha-L-iduronidase was retrieved from the Protein Data Bank (PDB ID: 3W81) to introduce the reported mutations and assess their impact on protein structure [8]. The mutation effects were evaluated using the DynaMut tool server, which provides insights into changes in protein stability and flexibility [9]. For the novel deletion mutation, a new 3D model was generated using the SWISS-MODEL server [10]. This model incorporated the deletion and was analyzed to determine the mutation's location within the protein structure and its potential effects on protein stability and substrate binding. By comparing the mutated and wild-type structures, we aimed to elucidate how these genetic alterations affect the overall function and interaction of the enzyme.

3. Results

3.1. Clinical features and IDUA activity

The clinical features of each patient and leukocyte IDUA activities are presented in Table 2. IDUA activities ranged from 0 to 0.39µKat/kg of protein with normal values between 1.7 and 3.5 µKat/kg of protein).

3.2. IDUA mutations analysis

We analyzed the IDUA gene of 4 MPS I patients from Tunisia. The affected probands in the three unrelated families proved the presence of two cases of Hurler phenotype, one case of Hurler / Scheie phenotype and one case of Scheie phenotype. We identified two distinct mutations associated with this disease. The first mutation, NM_000203.3:c.1598C > G in exon 11, was previously reported in the literature and was detected in patients 1 and 2 in homozygous sate. This mutation involves a substitution at codon 533, resulting in a proline-to-arginine change (p. Pro533Arg), which is known to be associated with pathogenic effects (Fig. 2).

The second homozygous mutation, NM_000203.3: c.1068_1088del (p.His356_Gln362del), identified in exon 8 of sibling pair (Patients 3–4), was described for the first time (Fig. 2). It results from a deletion of 21 nucleotides, corresponding to a loss of seven amino acids (His356 to Gln362), without causing a frameshift. Importantly, this deletion does not lead to a premature stop codon. Despite this, the removal of these amino acids may significantly affect the structure and function of the resulting protein, as the integrity of the region is altered.

Parental DNA samples were analyzed for the identified mutations through targeted sequencing. All parents showed heterozygous carrier status for their respective family's mutation, confirming autosomal recessive inheritance patterns.

Table 2
Results of biological data from patients studied.

Families	1	2	3	
Origins	Mahres, Sfax	Skhira, Sfax	Kairouan	
Patients	1	2	3	4
Phenotype	Hurler-Scheie	Scheie	Hurler	Hurler
Urinary GAGs	CS++ HS++ DS++	CS++ HS++ DS++	CS++ HS++ DS++	CS++ HS++ DS++
Hexuronic acid values in mg of acid glucuroniques/g of creatinine	47 Indicator: 12,20	17 Indicator: 10,89	–	–
Protein concentration (g/l)	3,50 Indicator: 4.86	3,75 Indicator: 3.25	4,56 Indicator: 2,8	4,73 Indicator: 2,74
Enzymatic activity of Hex (μkat/kg)	414 Indicator: 338	250 Indicator: 260	694 Indicator: 574	669 Indicator: 542
Enzymatic act of IDUA(μkat/kg)	0,39 Indicator: 15,58	0,30 Indicator: 15	0 Indicator: 3,32	0 Indicator: 2,37
Normal values	Hexuronic acid values in mg of acids glucuroniques/g of creatinine: 4.6–13 (3 to 7 years) Enzymatic act of Hex (μkat/kg): 244–730 Enzymatic act of IDUA (μkat/kg): 2,4–16			

GAGs: glycosaminoglycans; Act: activity; Hex: Hexosaminidases; IDUA: alpha-L-iduronidase; CS: Chondroitin sulfate; HS: heparan sulfate; DS: dermatan sulfate.

3.3. Bioinformatic finding

3.3.1. p.Pro533Arg mutation

In the present study, we have shown that the p.P533R mutation is located within the beta-sandwich domain, at a bend close to the helix involved in substrate binding (depicted as the red helix in Fig. 3a and b). This mutation results from the substitution of a small, neutral amino acid, proline, with a larger, positively charged arginine.

Proline's cyclic side chain restricts its flexibility, which is crucial given its proximity to the substrate-binding helix, where precise folding is required to maintain binding integrity. The introduction of a bulkier amino acid like arginine disrupts this delicate balance, as it occupies more space in the contact region between the beta-sheet and the substrate-bound helix. This likely reduces the protein's affinity for its substrate.

Additionally, the arginine substitution decreases flexibility and introduces a high positive charge, leading to steric clashes, as evidenced by crystallographic analysis. These clashes cause repulsion with neighboring amino acids, particularly those in the substrate-binding helix. Thus, the p.P533R mutation negatively impacts both the stability and activity of the protein. Indeed, the result of the variation on protein stability after the introduction of the reported mutation using DynaMut software showed that the mutated residue creates a rigidification of the structure which implicated in substrate binding (Fig. 3c).

3.3.2. p.His356_Gln362del mutation

Crystallographic analysis of the IDUA protein revealed that the amino acid sequence from His356 to Gln362 forms a crucial bend involved in substrate binding. This bend is located within the binding site and plays a critical role in maintaining proper substrate interaction. The residues Glu182 and Glu299 are part of the catalytic site, essential for enzymatic activity and located close to this deleted bend. The new mutation, which deletes these seven-amino-acid residues, removes the critical structure needed for substrate binding (Fig. 4a and b). Structural analysis using Swiss PDB Viewer® showed that the deletion disrupts the protein's conformation, particularly affecting the positioning of the two catalytic glutamic acid residues (Fig. 4c). This structural deformation leads to the elimination of the substrate binding site, resulting in a complete loss of enzymatic activity.

4. Discussion

Mucopolysaccharidosis type I (MPS I) is a rare lysosomal storage disorder with an incidence ranging from 0.69 to 1.66 per 100,000 newborns globally [11]. The prevalence varies by region, reflecting the influence of geographical and ethnic factors. In Saudi Arabia, it is estimated at 3.2 per 100,000 live births, compared to 0.69 per 100,000 in Germany and 1.33 per 100,000 in Portugal [12]. In Tunisia, MPS I incidence is also reported to be 3.2 per 100,000 births, largely attributed to the high rate of consanguineous marriages, which account for 32 % of all unions, and as high as 60 % in rural areas [5]. These marriages increase the risk of autosomal recessive diseases such as MPS I, especially with a relatively high incidence of 1.91 per 100,000 newborns, the highest recorded for any MPS subtype in Tunisia [13].

The clinical severity of MPS I ranges from the mild form (Scheie

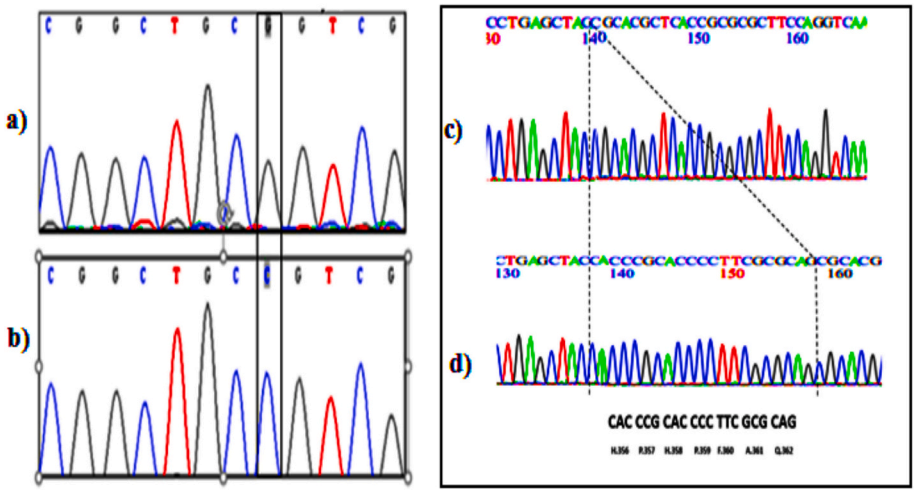

Fig. 2. Sequencing profile of the identified mutations p.Pro533Arg and p.His356_Gln362del in analyzed patients.
The left panel shows the electropherogram sequence of the reported mutation p.Pro533Arg in exon 11, detected in patients 1 and 2. The right panel illustrates the novel mutation p.His356_Gln362del in exon 8, found in patients 3 and 4. In the left panel, the top row **(a)** represents the normal control sequence, while the bottom row **(b)** corresponds to the homozygous mutation state. In the right panel the upper row indicates the homozygous deletion whereas the bottom the normal control.

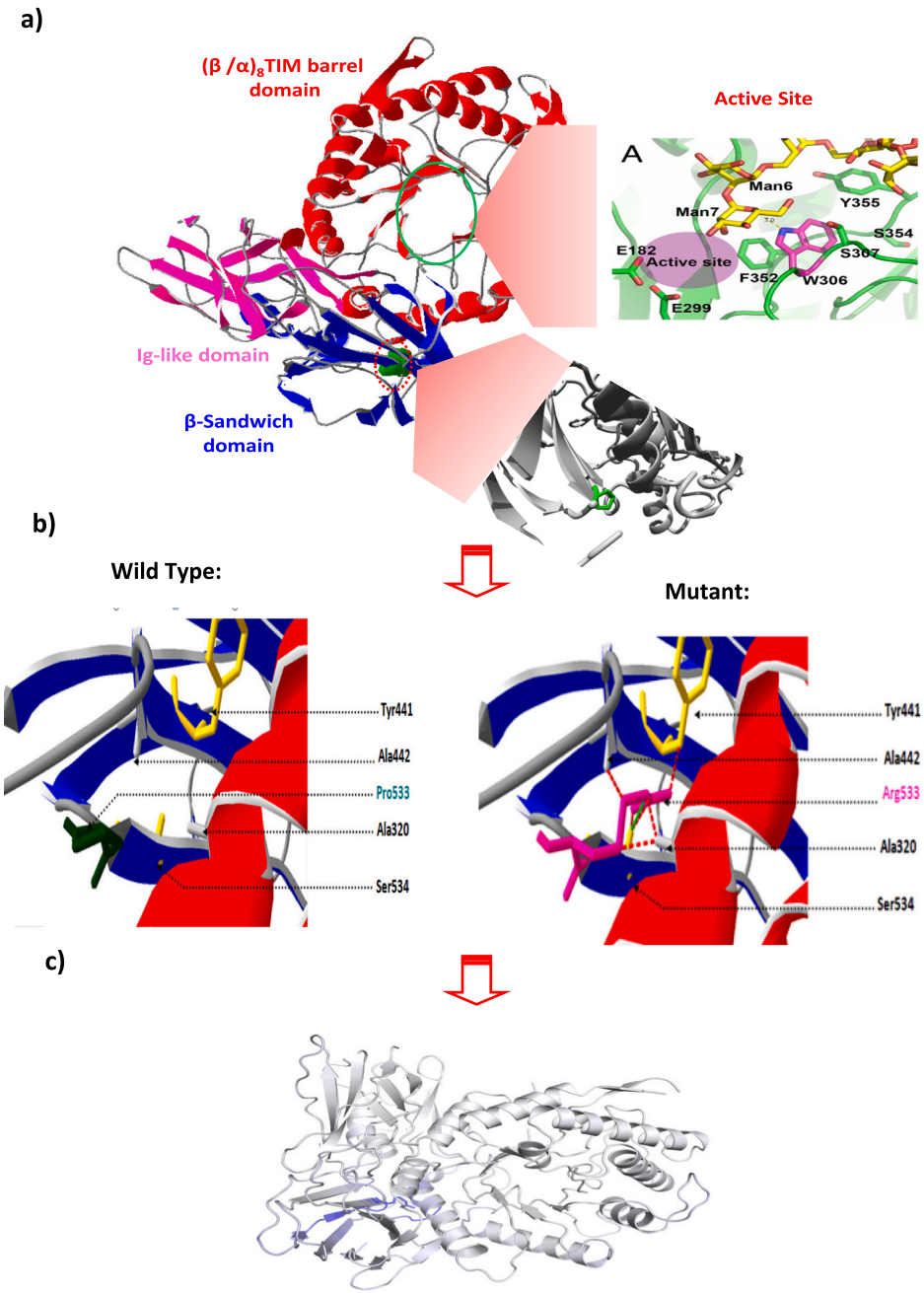

Fig. 3. Crystallographic structure analysis of the Human alpha-L-iduronidase.
(a): the IDUA protein is composed of 3 domains: "TIM BARREl"domain which is composed of 8 alpha helices surrounding 8 beta sheets forming a barrel structure. The catalytic site is located almost in the middle of this domain. The second domain is a beta sandwich domain which is made up of antiparallel beta sheets that come together to form a structure resembling that of a sandwich. The third domain "IG like domain", which has a structure very similar to that of immunoglobulin. The mutant residue P533 is located in the beta sandwich domain at a bend very close to the helix involved in substrate binding (red helix). **(b)** The mutant residue Arg533 alters its interactions with neighboring residues by introducing a negatively charged side chain, which leads to the formation of new covalent interactions. This change in charge disrupts the original molecular interactions and promotes novel bonds with nearby residues. **(c)** The result of the variation on protein stability after the introduction of the reported mutation p.Pro533Arg using DynaMut software. The amino acids are colored according to the vibrational entropy change upon mutation. Blue represents a rigidification of the structure while the red color indicates a gain in flexibility where present.

syndrome) to the severe form (Hurler syndrome), with the intermediate Hurler-Scheie syndrome also recognized. The incidence of Scheie syndrome varies between 1 in 115,000 and 500,000 live births, while Hurler syndrome occurs in about 1 in 100,000 [14]. Molecular analysis of the IDUA gene, responsible for MPS I, has identified more than 300 mutations, including the commonly encountered p.Q70X and p.W402X mutations in the Caucasian population, and the p.P533R mutation prevalent in North African populations [5].

In this study, we analyzed four patients including two siblings from three unrelated Tunisian families, all of whom were born from consanguineous unions. This pattern is consistent with previous studies on MPS I, demonstrating a clear link between consanguinity and the occurrence of autosomal recessive disorders. Socioeconomic and cultural factors drive this high level of consanguinity in Tunisia, often resulting in a higher incidence of genetic diseases. A notable example is the frequent occurrence of the p.Pro533Arg mutation in both Tunisian and Moroccan

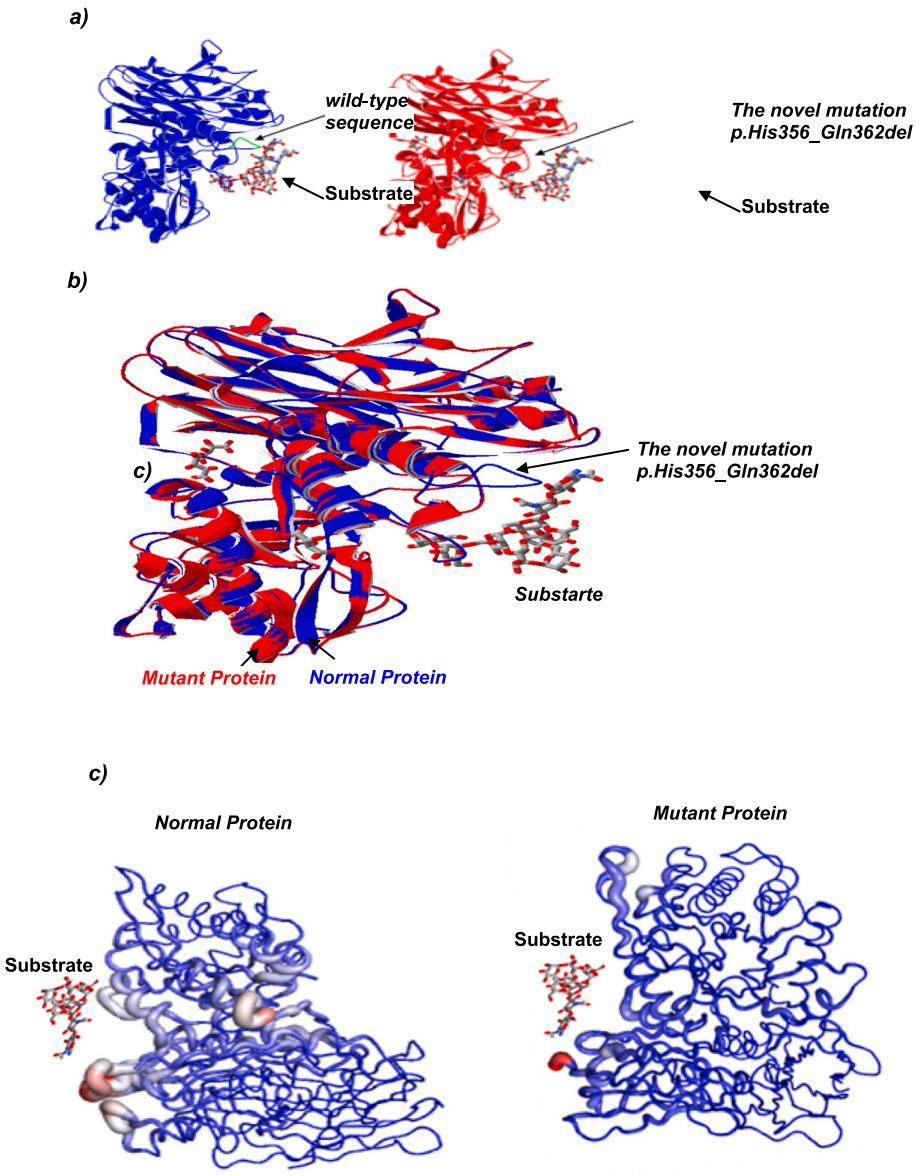

Fig. 4. Structural Analysis of Alpha-L-Iduronidase showing the Impact of the p.His356_Gln362del Mutation on Protein Conformation and Stability.
(a) Three-dimensional structure of the normal alpha-L-iduronidase protein and the alpha-L-iduronidase-His356_Gln362del variant, modeled using the SWISS-MODEL server based on structural homology.
(b) Superposition of the normal alpha-L-iduronidase (blue) and the alpha-L-iduronidase-His356_Gln362del variant (red), highlighting structural differences between the two proteins.
(c) Deformation magnitude caused by the p.His356_Gln362del mutation, as predicted by the DynaMut® online server. The scale represents weak deformation in blue, moderate deformation in white and intense deformation in red.

populations, where recent studies suggest a common founder origin [5].

The clinical presentation of MPS I is highly variable, with symptoms progressing in severity over time [14]. In our study, patients 3 and 4, siblings from family 3, both presented with Hurler syndrome, characterized by mental retardation, facial dysmorphia, corneal opacity, hepatosplenomegaly, and skeletal deformities. These symptoms are consistent with the severe phenotype observed in Hurler syndrome, with survival not exceeding 10 years [15]. Conversely, patient 1 presented with the intermediate Hurler-Scheie phenotype, characterized by mental retardation and variable survival, while patient 2 exhibited the milder Scheie syndrome, with normal survival and no significant mental impairment [16]. This variation in clinical presentation reflects the diverse phenotypic spectrum of MPS I.

The p.P533R mutation, identified in patients 1 and 2, is a previously described missense mutation [17]. The structural impact of this mutation has been minimally studied, leaving gaps in our understanding of how it affects the protein's folding and function. Crystallographic studies showed that the proline residue is located near the helix involved in substrate binding. The replacement with arginine, which is bulkier, likely disrupts the contact between the beta-sandwich domain and the substrate-binding helix. This disruption is thought to reduce substrate affinity and impair enzymatic activity [17]. Despite this, residual catalytic activity remains, which explains the intermediate and mild phenotypes observed in patients 1 and 2 [17].

The p.His356_Gln362del mutation, discovered in patients 3 and 4, involves a 21-nucleotide deletion in exon 8, leading to the loss of seven amino acids (His356 to Gln362). This reported mutation was absent in population databases (gnomAD, 1000 Genomes, Iranome) and Tunisian controls, confirming its pathogenic rarity. While small in-frame deletions represent $\leq$5 % of IDUA mutations, the 7-amino-acid deletion

described in this study represents the largest reported microdeletion. The variant's location in exon 8, a known mutational hotspot, further supports its clinical significance.

Furthermore, the deleted region forms a critical bend essential for substrate binding and is located near the catalytic residues Glu182 and Glu299. The deletion disrupts the substrate-binding site, resulting in a complete loss of enzymatic activity. The severe clinical outcomes in patients 3 and 4, both of whom died at a young age, correlate with the loss of this functional region.

Given the high rate of consanguineous marriages and the prevalence of the p.P533R mutation in Tunisia, genetic counseling and family studies are critical [5]. The identification of common mutations like p.P533R can guide initial screening efforts, followed by comprehensive IDUA gene sequencing if this mutation is absent. In this study, the third family underwent prenatal testing after the death of two affected children, which confirmed that the fetus was heterozygous for the p.His356_Gln362del mutation. Such early identification offers families the opportunity for informed decision-making regarding future pregnancies.

Current therapeutic options for MPS I, such as bone marrow transplantation and enzyme replacement therapy (ERT), alleviate some systemic disease manifestations without addressing the neurological complications [18–21]. In Tunisia, these treatments are not widely accessible due to financial limitations, leaving patients reliant on symptomatic management. Genetic testing thus becomes a key tool, offering families the possibility of prenatal diagnosis and appropriate genetic counseling. In our study, none of the patients received bone marrow transplantation or ERT, underscoring the need for improved access to these therapies in Tunisia.

An emerging therapeutic approach for MPS I, particularly for patients harboring missense mutations like p.P533R, is the use of pharmacological chaperones. These small molecules are designed to bind and stabilize misfolded proteins, facilitating their proper folding and enhancing their function. In the case of the p.P533R mutation, the structural disruption caused by the substitution of proline with arginine leads to protein misfolding, resulting in decreased enzymatic activity. Chaperones can correct this misfolding by binding to the mutated IDUA protein, stabilizing its conformation, and restoring partial enzymatic function. This therapeutic strategy holds promise, especially for patients with residual enzyme activity, as seen in those with intermediate or mild phenotypes [22,23].

The importance of structural analysis in understanding the effects of mutations like p.P533R cannot be overstated. By using crystallographic and computational tools to model the mutated protein, we can identify specific defects in folding and substrate binding. This detailed understanding allows researchers to design targeted interventions, such as chaperone therapies, aimed at correcting these structural abnormalities. Therefore, structural studies are critical not only for understanding the molecular mechanisms underlying the mutations but also for guiding the development of novel treatments.

5. Conclusion

In conclusion, our study emphasizes the importance of early diagnosis and intervention in MPS I. We recommend routine screening for urinary GAG levels in patients presenting with unexplained dysmorphic features or growth delays. Additionally, a multidisciplinary approach involving clinicians, geneticists, and pharmacists is essential to ensure optimal care. Public awareness campaigns on the risks of consanguineous marriages, especially in families with known cases of MPS I, are also necessary. Finally, antenatal diagnosis should be promoted in families at risk to prevent the recurrence of this debilitating condition.

Availability of data and materials

For Data evaluation please contact Dr. Amri Yessine at; amri.yessine@yahoo.com

CRediT authorship contribution statement

Mariem Rebai: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Yessine Amri:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Data curation. **Chaima Sahli:** Writing – review & editing, Visualization, Validation, Methodology, Formal analysis. **Hajer Foddha:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Taieb Messaoud:** Writing – review & editing, Validation, Methodology. **Hela Boudabous:** Writing – review & editing, Investigation, Conceptualization. **Hassen Ben Abdennebi:** Writing – review & editing, Methodology, Investigation. **Salima Ferchichi:** Writing – review & editing, Supervision, Investigation. **Latifa Chkioua:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Consent to publication

Written informed consent was obtained from the parents or legal guardians of the patients for their participation and publication of this work. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Ethics approval and consent to participate

The families gave informed consent before with drawal of blood samples and written informed consent was obtained and signed by all MPS I and controls families, in addition the verbal consent was also obtained during consultation. The study was approved by the ethics committees for scientific research of the La Rabta Hospital Tunis, Tunisia. All procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 and approved by the Ethics Committees of the respective Tunisian hospitals.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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